

Separation of compounds by paper chromatography

INTRODUCTION

Chromatography is the physical separation of components of a mixture on the basis of their differing affinities for a stationary phase vs. a mobile phase. It is one of the most common chemical and biochemical separation techniques. In a variety of chromatography methods, chemists use liquid solvents moving compounds over paper, thin film, or materials held in a column. The material in the column can be a solid, a gel, or a polymer. We can analyze the compounds as they come out of the column (at different times) or remove the compound from the paper and then analyze it. In gas chromatography (GC), a mixture is volatilized and carried through materials held in a column by a carrier gas. The components of the mixture are then analyzed the separated gases as they come out.

Paper chromatography (PC) is a technique similar to the widely-used thin-layer chromatography (TLC). In typical thin-layer chromatography, silica gel – a material commonly used as a stationary phase – is coated onto a glass or plastic sheet. In paper chromatography, the mixture is spotted onto a sheet of paper. Both of these techniques can be used to help to identify an unknown in solution.

Paper chromatography works because of the capillary action of water, or solvent, in paper. You may have noticed a towel hanging into the bath tub, with one end in water and the other end out of the water on the floor. Water travels through the towel, forming a puddle on the floor. Capillary action or the affinity of water for the towel allows the water to travel through the towel. Think of a stain caused by an uncapped pen in a pocket. Was there one spot or rainbow streaks? Different dyes in the pen ink travel at different rates as capillary action pulls the material through the cloth at different speeds. In this experiment you will put a paper spotted with unknown and known solutions into a jar with a small amount of a solvent. You will see the solvent travel up the paper, beyond the level of the liquid in the jar. The attraction of the solvent for the paper is stronger than the force due to gravity.

Capillary action allows us to separate compounds due to the attraction, or affinity, of the compounds for the mobile phase (the solvent) vs. the stationary phase (the paper or thin film). Compounds which are not soluble in the solvent or are very attracted to the stationary phase stay in place. Compounds that are very soluble and less attracted to the stationary phase travel up the stationary phase with the solvent. Some compounds are somewhat soluble and somewhat attracted to the stationary phase. They travel up the paper, but at a slower rate.

Mobile phase: This is the solvent. It may be a single compound or a mixture of solvents, often with different polarities.

Stationary phase: This is the chromatographic medium – in our experiment, the paper.

Baseline (origin): This is where you put the mixture of compounds on the chromatography paper. Generally, you will make a pencil line to show you where you put the mixtures. The baseline must be above the liquid level, so that the solvent does not dissolve the compounds into the liquid phase in the chamber.

Solvent front: This is the highest point that the solvent traveled during “developing”. **Note:** you need to mark where the solvent front reaches before the solvent evaporates.

Retention factor (R_f): This is the ratio of how far the solute (metal ions) travels on the chromatograph vs. how far the solvent front travels. The R_f is a property characteristic of a substance (intensive property) and will be the same if the solvent and stationary phase are the same.

$$R_f = \frac{\text{distance traveled up the paper by the compound (baseline to spot)}}{\text{distance traveled by the solvent (baseline to solvent front)}}$$

In this experiment, you will analyze the characteristics of five known metal ions on a paper medium. You will then compare 3 unknown solutions to these known ions to determine what metal ions are present in them.

SAFETY and DISPOSAL

You are working with acetone, hydrochloric acid and ammonium hydroxide. No open flames are allowed near the eluting tanks. The acetone mixture is returned to a waste container inside the hood. The ammonia solution can go down the drain with lots of water. Use the concentrated ammonia solution in the hood only, as the fumes are irritants. Use precautions working with acids, bases and solvents.

PROCEDURE

A. Prepare toothpicks for spotting the paper and the spot plate.

Use fine toothpicks to spot the paper. You can break or cut the toothpicks to make them thinner, so that they produce a fine spot. You will be putting a drop of each known and unknown onto a spot plate for this experiment. Clean a spot plate and label it so you know which compounds are in which spot (using wax pencils or markers)

Metal ion separation.

Prepare the stationary phase:

This is the chromatography paper – about 10 x 20 cm. Handle the top (long) edge only and place the paper onto a clean sheet of binder paper or paper towel, not on the lab bench. Draw a pencil line 1.5 cm from the bottom (long) edge and space out the spots evenly on the paper, starting no less than 3 cm from the short edge. Label the identity of these spots (with pencil), so that you can remember what is at each position. Put the 5 knows and 3 unknowns onto the same paper, so they can easily be compared.

Prepare the metal ion eluting chamber:

Use a clean 600-1000 mL beaker. Use plastic wrap for a cover. Carefully pour 10 mL of the prepared eluting solution into beaker. You can pour the solution down a stirring rod to avoid splashing the solution onto the walls. The prepared eluting solution is a mixture of 9 parts acetone to 1 part 6M HCl. Leave the plastic wrap over the beaker except when inserting or removing the chromatograph. The cover will prevent evaporation of the acetone. It's better for your health and it allows the chromatograph to elute with a constant concentration of eluting solution.

Carry out the separation and analysis for the metal ions:

Clean and dry a ceramic or plastic well plate and label it for each of your known and unknown solutions. Put 2-3 drops of each solution into its labeled well. Using the toothpicks you prepared earlier, practice spotting these liquids onto scrap chromatography paper before you spot your stationary phase. You want to make 2-3 mm wide spots. Very small spots are hard to read and large spots tend to run together, once developed.

Once you are comfortable with the procedure, place the stationary phase onto a piece of binder paper (to protect it from any contaminants on the bench) and spot the stationary phase with the five knowns and the three unknowns. Dry the stationary phase under the heat lamps and spot again. Be careful - the heat lamp can burn the paper, or cause the compounds in the mixtures to react. Be sure the spots are dry before proceeding. Spot each compound onto the stationary phase 2 additional times, taking care to keep the spots small, and drying in between.

Roll the chromatograph into a loose cylinder and fasten at the top, middle, and bottom with staples. Be sure there is a small gap between the two edges of the paper, or the paper will not elute correctly.

Be sure that the developing tank is located in a quiet place on the lab bench. Put the cylinder into the developing tank. The entire bottom of the cylinder must sit in the liquid, and the cylinder must not touch the edge of the tank. Replace the plastic wrap and wait until the solvent front has moved within 1.5 cm of the top. Do not disturb the tank while it elutes.

Once the solvent front is near the top of the paper, remove the stationary phase and quickly mark the solvent front. Set the cylinder onto clean paper so that you can visually examine the spots (do not flatten it yet). Let the solvent evaporate off of the paper (you can put the cylinder under a heat lamp to aid this).

Some compounds will not be visible. To enhance the visibility, we will use an ammonia chamber to allow ammonia gas to complex with the metal ions.

Preparing the metal ion ammonia chamber:

Safety note: The ammonia chamber should be prepared and left in the fume hood. Avoid breathing ammonia fumes. The purpose of the ammonia chamber is to help visualize the metal ions on the paper after elution. Note that one ammonia chamber can be used more than once, so please share with other students when you are finished with yours. Put approximately 5 mL of concentrated ammonium hydroxide into a 20-50 mL beaker. Position the small beaker in the center of a clean, dry 1000 mL beaker. Cover the ammonia chamber with plastic wrap. Leave the plastic wrap over the beaker at all times, except when inserting or removing the chromatographic paper. Note also that ammonia reacts with HCl from the eluting solution, producing ammonium chloride. This white powder will not affect the results.

Put the chromatograph (still in a cylinder form) into the ammonia chamber and replace the plastic wrap. Wait at least 20-30 minutes or until the spot for each known compound is visible and 2-3 spots for each unknown are visible.

Remove the chromatograph from the chamber, unroll it, and place it onto a clean piece of paper. **Use a pencil to circle all spots (some disappear quickly).** Also draw a line to mark the solvent front (if you did not already mark it properly before). Record the spot color. Analyze your chromatograph. Measure the distance of each spot above the baseline (d_{spot}) and record the distance from the baseline to the solvent front ($d_{\text{solvent front}}$). Be careful to look closely and find all of the spots in the unknown. You will not know precisely how many compounds are in each unknown.

Determine the composition of your unknowns, based on spot color and R_f . See the Data Analysis section following for suggestions on organizing your data.

Several cautions: In this lab, you will be monitoring a series of inorganic compounds. Some of these compounds can be present on your lab desk and the oils and salt on your hands can confuse the results. You will be using toothpicks to spot the paper. These can hold a lot of liquid, making a very large spot. You will need to dry the paper, using a heat lamp, between spotting (spot/dry/spot/dry...). The chromatography paper should not touch any walls of the developing tank. It results in confused data.

Put chromatography paper onto clean paper for spotting the compounds onto the paper

Only touch the edges of the paper.

The heat lamp can burn the paper if it gets too close.

DATA ANALYSIS

In your notebook, draw a picture of your chromatogram. You will turn in your metal ions chromatogram with your lab report. For each individual spot on the chromatogram, you need to measure the distance from the baseline to the solvent front and from the baseline to the spot (use the middle of the spot unless the spot is *on* the baseline or the solvent front). You must then calculate the R_f values for each spot. Show calculations (may show R_f calculation on the table).

Sample Data Table:

Solution	Spot color		d_{spot} (cm)	$d_{\text{solvent front}}$ (cm)	R_f (may show calc in the table)
	After Development				
Metal 1					
Metal 2					
Metal 3					
Metal 4					
Metal 5					
Unknown A, spot 1					
Unknown A, spot 2					
Unknown A, spot 3					
Unknown B, spot 1					
Unknown B, spot 2					
Unknown B, spot 3					
Unknown C, spot 1					
Unknown C, spot 2					
Unknown C, spot 3					

For each metal (1-5) clearly indicate what the known ion in the solution is (eg. Fe^{3+})

Results:

Clearly state what metal ions you have determined to be present in each of your unknowns.

Paper Chromatography Experiment • Assignment & Report Guidelines

READING Experiment – Lab Manual Pages / Handout
Chemistry, 5th ed. by Silberberg: Section 2.9 including “Tools of the Laboratory”

PRE-LAB Begin the prelab on a new page of your laboratory notebook. **ALL elements of the pre-lab MUST be completed before an experiment is started.** The COPY page from your notebook will be collected as you enter the lab. The original pages must stay in your notebook.

Heading

- Title of experiment and number, your name, the dates of the experiment.

Purpose

- Briefly, but specifically explain the purpose of this experiment.

General Strategy

- **Summarize** the steps of the experiment, and **explain** how you will use the information in order to achieve the goal(s) in the purpose.

Answers to Pre-Lab Questions

- Answer the questions on the PRE-LAB Questions page in the lab manual. Write all answers on that page and turn in with the COPY pages of the pre-lab information in your notebook. Show ALL units and work.

MINI LAB REPORT *Begin the lab report section on a new page of the lab notebook.*

Heading

- Title of experiment and number, your name, the dates of the experiment.

Data / Observations / Calculations/ Results

- Prepare a data table using the table in the lab manual pages as an example (start this on a new page).
- After visualization in the ammonia, draw at full size your paper chromatograph(s) in your notebook.
 - Measure the appropriate distances on the original chromatograph and record in the data table.
 - Show your original chromatograph and your drawing to the instructor to get your data signed.
- Results – Calculate R_f values (and record in the data table).
- State the unknown numbers and identify all metal cations in each unknown respectively.

Discussion Questions

The discussion question responses may be word-processed or written in the lab notebook. Please restate the questions as you give the answer.

1. Briefly describe how paper chromatography works with the unknown mixtures: How do the stationary phase (paper), eluting solution, and the metal ions to be separated interact?
2. What eluting solution was used for the experiment? How would changing this system change the experiment?

5. Anya ran a second chromatogram data and made the following chart. Make a table to describe her data – include distance spot traveled, distance of the solvent front, and R_f . State the composition of his unknowns. The knowns are 1,2, and 3. State which compounds are in unknowns A and B. Give the R_f values for 1-3 and all of the compounds (spots) in unknowns A and B:

